REMARKS

In the foregoing amendments, editorial changes were made to applicant's claims, such as rewriting the preamble of applicants to read: "A black currant anthocyanin-containing food composition suitable for consumption by humans." This aspect of applicants claimed invention are supported in the present specification disclosure at page 30, line 6, to page 31, line 3; page 34, last 7 lines; page 37, last 10 lines; and elsewhere. In addition, Claims 37 and 38 were limited by amendment that will be discussed below. Claim 41 was added to the application, which is a combination of claims 27 and 30. Accordingly, claims 27-30 and 32-41 are present in the application for consideration by the examiner.

Applicant greatly appreciates the courtesies extended the undersigned by Examiner Jagoe in a personal interview on November 18, 2004. In the personal interview, the following items 1-6 were discussed:

(1) Applicant was informed that claims 35-40 would be objected to as not imparting any further physical or otherwise material limitation to the composition of claim 27. Applicant's representative was advised that these claims could be examined in a divisional application as method of use claims.

(2) Applicant was advised to put the limitations of claim 30 into claim 27 to further distinguish the composition over fruit juice.

(3) The examiner was unclear what is meant by "on the basis of solid matters." The examiner questioned: Is this a w/v or v/v?

(4) Applicant was advised to change "absorb" to "adsorb" in claim 33 as per the specification on page 19.

(5) Japanese prior art from page 5 of the instant specification was discussed during the interview, regarding the black currant juice extracted by chemical methods.

(6) Applicant's representative discussed differences over Lawhon *et al.* (Lawhon). Lawhon has a two-step process versus the one-step process of the instant application.

With respect to item (1) above, applicant respectfully submits that claims 35-36 further define that the composition of claim 27 has a particular form, namely, a health-promoting food or drink which comprises the composition according to claim 27. For this reason, applicant respectfully submits that these claims further define claim 27 and, thus, are proper claims. Claims 37-40 define a composition containing "an effective amount" of the black currant anthocyanin for improving specific human conditions. In the foregoing amendment, claims 37 and 38 were limited to define "an effective amount" of the black currant anthocyanin "for improving visual function of alleviating asthenopia and/or improving adaptation to darkness." The effect of alleviating asthenopia is shown in example 3 beginning at the bottom of page 34 the present specification. The effect of improving adaptation to darkness is shown in example 4 beginning at the middle at page 37 of the present specification. The use of language, such an "effective amount" including the desired property or effect has long been recognized as acceptable in U.S. patent practice. *In re Halleck*, 164 USPQ 647, 57 CCPA 954 (CCPA 1970).

Considering item (2), the examiner stated that a product-by-process claim that combines the limitations of claims 27 and 30 may have the best possibility of being patented. In the foregoing amendment, new claim 41 was added to the application that combines the limitations of claims 27 and 30 in a

product-by-process claim. Favorable consideration and allowance of claim 41 are respectfully requested.

In item (3) the examiner stated that it is not clear what is meant by "on the basis of solid matters." The examiner questioned: Is this a w/v or v/v? In the present claims, the expression "on the basis of solid matters" is used in a manner typical in this art. The expression 5 to 25% by weight on the basis of solid matters means that if a composition contains 100 grams of solid matters (the solid matters including those dissolved and partially dissolved and those not dissolved), 5 to 25 grams of the solid matters are black currant anthocyanin.

In the foregoing amendments, claim 33 was amended by changing "absorb" to "adsorb" as set forth in applicant's specification on page 19 and as suggested by the examiner at the personal interview in item (4).

The examiner mentioned the Japanese prior art discussed on page 5 of the present application in item (5). In this discussion, anthocyanin was purified. Applicant's specification does not state the concentration of anthocyanin in the purified product. However, this portion of applicant's specification explains that the product obtained by the Japanese prior art cannot be used as a food, because it contains organic media such as butanol and amyl alcohol. Applicant's claims define a food composition suitable for human consumption. For this reason applicant's claims define subject matter that is patently distinguishable from the Japanese prior art discussed on page 5 of the present specification.

It is well established in the case law that if it is determined that limitations in the preamble of a claim are necessary to give meaning to the claim and properly define the invention, then such limitations must be considered when determining the patentability of the claims. The predecessor court of the Court of Appeals for the Federal Circuit (CAFC), namely, the Court of Custom and Patent Appeals (CCPA) summarized this approach in *Kropa v. Robie,* 88 USPQ 478 (1951), after reviewing some 37 cases that turned on the limiting nature of the preambles to the claims in suit. See also *Loctite Corp. v. Ultraseal Ltd.*, 228 USPQ 90, 94 (Fed. Cir. 1985). According to the court in *Kropa*:

> the preamble has been denied the effect of a limitation where . . . the claim or [interference] count apart from the introductory clause completely defined the subject matter [of the invention], and the preamble merely stated a purpose or intended use of that subject matter. On the other hand, in those . . . cases where the preamble to the claim or count was expressly or by necessary implication given the effect of a limitation, the introductory phrase was deemed essential to point out the invention defined by the claim or count. In the latter class of cases, the preamble was considered necessary to give life, meaning and vitality to the claims or counts.

Examples of preambles cited in *Kropa* as expressly or impliedly held to express a limitation in the claims are "An insecticide," "An insecticide composition," "In a tire," and "An expansible diaphragm." Applicant respectfully submits that the claims in this application present precisely the situation where the preamble of a claim has been held to express a limitation in the claim in *Kropa.* In the prior art, black currant anthocyanin-containing food compositions suitable for human consumption, which comprise 5 to 25% by weight of black currant anthocyanin, were not available. The preamble of

appellant's claims distinguishes the presently claimed "black currant anthocyanin-containing food composition suitable for consumption by humans" from other types parts of black currant anthocyanin-containing composition that are not suitable for human consumption.

In item (6) of the personal interview, the prior art cited against applicant's claims was discussed. The differences between the presently claimed invention and the teachings cited against applicant's claims are discussed below in applicant's traversal of the prior art rejections.

In the final Office action mailed November 25, 2003, claims 39 and 40 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Official action stated that the phrases "improving blood fluidity" and "lowering blood pressure" in these claims are relative expressions that render the claims indefinite. The applicant has difficulty understanding this position.

Applicant respectfully submits that persons skilled in the art precisely understand the meaning of the expressions "improving blood fluidity" and "lowering blood pressure." For example, after a mammal (human) consumes the composition, food, or drink according to claims 39 and 40, its blood fluidity will be improved relative to its blood fluidity prior to taking the composition, food, or drink. Similarly, a mammal (human) consuming the composition, food, or drink of claims 39 and 40 will have a lower blood pressure relative to its blood pressure prior to consuming the composition food or drink. In

addition, applicant respectfully note that the present specification disclosure discusses blood fluidity improvement and blood pressure lowering of the compositions of applicant's claims at page 7, first complete paragraph; page 12, first complete paragraph; the paragraph bridging pages 28 and 29; the first two complete paragraphs on page 29; and elsewhere. For all these reasons, applicant respectfully submits that one of ordinary skill in the art would attach a particular and definite meaning to the expressions "improving blood fluidity" and "lowering blood pressure," as set forth in claims 39 and 40.

In order to expedite the allowance of the present application, claims 39 and 40 were amended in the response after final while the on April 23, 2004, to recite "improving... compared to... before the ingestion of the composition." For all the foregoing reasons applicant respectfully submits that claims 39 and 40 particularly point out and distinctly claim the subject matter regarded as the invention within the meaning of 35 U.S.C. § 112, second paragraph. Therefore, applicant respectfully requests that the examiner reconsider and withdraw this rejection.

In the final Office action mailed November 25, 2003, no specific prior art rejections of applicant's claims were set forth. In a previous Office action mailed April 10, 2004, claims 8-12 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. patent No. 4,643,902 of Lawhon *et al.* (Lawhon); and claims 1-7, 13-14, and 18-25(26) were rejected under 35 U.S.C. § 102(b) as being anticipated by, or in the alternative, as obvious under 35 U.S.C. § 103(a) over Lawhon in view of Nakhmedov *et al.* (Koservanaya I Ovoshchesushil'naya

Promyshlennost) and British patent specification number 1,007,751 (British '751). In applicant's response filed on September 9, 2003, claims 1-26 were canceled and new claims 27-40 were added to the application. The final Office action does not identify which claims are rejected. Based on the subject matter in the new claims compared to the original claims, it is assumed that claims 30-34 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Lawhon, and that claims 27-29 and 35-38 were rejected under 35 U.S.C. § 102(b)/35 U.S.C. § 103(a) over Lawhon in view of Nakhmedov and British '751. The subject matter of claims 39 and 40 does not correspond to a previously presented claimed. Therefore, the applicant cannot determine how (or if) these claims were rejected over the previously cited prior art. The examiner's clarification in this regard is respectfully requested.

At the bottom of page 3 the Official action stated that the rejection over Lawhon was maintained. In response to applicant's argument that Lawhon does not contemplate or suggest a "charged" reverse osmosis membrane, the Official action noted example 4 in column 10 of Lawhon, as proposing the use of a membrane having a 99% rejection for NaCl. The Official action apparently interpreted this portion of Lawhon as meaning or requiring that membranes having a 99% rejection for NaCl must necessarily be a "charged" reverse osmosis membrane. In other words, appears to be the position of the Patent Office that in order for a membrane to have a 99% rejection for NaCl, it must be a charged reverse osmosis membrane. Applicant respectfully submits that such reasoning is not correct. In order to maintain such a position, applicant

respectfully submits that a teaching reference in support this position must be set forth. In addition, it is respectfully noted that uncharged reversed osmosis membranes can have a retention rate of 99% in the case of NaCl, which is discussed in the first complete paragraph on page 19 of the present specification. Accordingly, applicant respectfully submits that reasoning the forth in the Office action is fundamentally flawed and the teachings of Lawhon cannot possibly contemplate or suggest the use of a charged reversed osmosis membrane, as required in the present claims.

In more detail, it is respectfully noted that the Official action stated that the reverse osmosis membrane used by Lawhon is aided by charged particles. However, the charged reverse osmosis membrane used in the presently claimed invention is structurally and functionally different from the reverse osmosis membrane proposed by Lawhon, and therefore the product obtained by the method using the charged reverse osmosis membrane in applicant's claims is necessarily different from the product obtained by the Lawhon's process.

In the presently claimed process, the charged reverse osmosis membrane is used to separate anthocyanin from other components, such as sugars and acids, and to concentrate the anthocyanin to 1% or more on the basis of solid matters. The molecular weight fractionated by the charged reverse osmosis membrane used for the present process is generally 3,000 to 5,000. On the contrary, the reverse osmosis membrane used in Lawhon fractionates molecules of which molecular weight is a little larger than a water molecule. Accordingly, the charged reverse osmosis membrane of the present claims is

different from the reverse osmosis membrane of Lawhon and, therefore, the components obtained by the charged reverse osmosis membrane of the present claims is different from those obtained by reverse osmosis membrane disclosed in Lawhon.

The charged reverse osmosis membrane is used to purify solid materials from a solution. The present specification describes that the salt retention rate of the charged reverse osmosis membrane of applicant's claims is 5 to 20%. The salt retention rate means the amount of salt which is retained in a reverse osmosis retentate. That is, "the salt retention rate of 5 to 20%" means that 5 to 20% of salt is retained in the retentate and 80 to 95% of salt is permeated in the permeate. On the contrary, since reverse osmosis membrane as used within the teachings of Lawhon is mainly used for the specified purpose of, for example, to prepare pure water from sea water, the salt rejection rate means the rate of the rejection of salt in the permeate. Therefore, 99% of salt rejection rate of the reverse osmosis membrane in Lawhon means that 99% of salt is retained in the retentate. Therefore, even in view of the salt retention, the charged reverse osmosis membrane of the presently claimed invention is significantly different from the reverse osmosis membrane disclosed in Lawhon.

Consequently, the charged reverse osmosis membrane of the present claims is different from the reverse osmosis membrane of Lawhon in properties. By using the charged reverse osmosis membrane of the present claims, the two fractions (i) and (ii) below were obtained (See Figure 1 attached hereto, which illustrates the process of both of the present invention and that of Lawhon.).

(i) reverse osmosis retentate including anthocyanin and other high molecular weight components

(ii) reverse osmosis permeate including flavor and aroma components, sugars, acids, water and salts

Since the molecular weight of anthocyanin is about 500, one of ordinary skill in the art would expect that anthocyanin would be included in the reverse osmosis permeate. However, unexpectedly and surprisingly, anthocyanin is included in the reverse osmosis retentate. This unexpected and surprising property of the charged reverse osmosis membrane is used in the invention of applicant's claims. In the process of the presently claimed invention, anthocyanin can be unexpectedly and surprisingly separated from flavor and aroma components, sugars, acids, water and salts and therefore purified by a process including use of a charged reverse osmosis membrane.

On the contrary, the process proposed by Lawhon is concerned with a process to produce fruit juice -- not to purify anthocyanin as presently claimed. In the conventional process to produce fruit juice, whole fruit juice is heated for sterilization of microorganism included in the fruit juice. However, the sterilization also reduces the flavor and aroma components. In Lawhon's process, an ultra filtration (UF) membrane is used to inhibit the reduction of the flavor and aroma components. In the process, fruit juice is fractionated into a retentate and permeate by using the UF membrane. The retentate includes microorganisms to be deleted as well as high molecular weight components and sugars. The permeate includes anthocyanin, sugars, acids,

flavor and aroma components. The retentate only is heated for sterilization. The permeate is not heated since it never includes microorganisms to be deleted. After the retentate is heated, it is mixed with the permeate to produce a fruit juice containing no microorganism. Furthermore, on one embodiment of the Lawhon's process, the UF permeate is further subjected to reverse osmosis membrane separation and the flavor components and aroma components are concentrated into the retentate.

Lawhon proposes in its explanation of reverse osmosis membrane that the semipermeable membrane is used to make a solvent (in case of juice, water) pass through and retain other components such as anthocyanin, sugars, acids, flavor and aroma components. This is demonstrated by tables 4 and 5 which indicates that the reverse osmosis permeate does not include solid components and sugars. In the Lawhon's process, a juice is separated into 3 fractions (a) to (c) below by UF membrane treatment followed by reverse osmosis membrane treatment (See Figure 1 attached hereto.).

(a) UF retentate including enzymes and microorganisms to be inactivated and sterilized

(b) reverse osmosis retentate from UF permeate, including flavor components, aroma components, sugars and acids

(c) reverse osmosis permeate of UF permeate mainly including water and including little solids

Accordingly, in the process proposed by Lawhon, the important anthocyanin is mainly present in (b) above and partially present in (a) above.

Thus, in the process proposed by Lawhon, fractions (a) to (c) are mixed to obtain general juice as obtained by squeezing fruits as indicated in Fig. 1. Alternatively, fractions (a) and (b) only are mixed to obtain concentrated juice and fraction (c) is discarded.

As described above, anthocyanin is included in retentate of reverse osmosis membrane together with sugars and acids and is not never purified in the process proposed by Lawhon. On the contrary, in the presently claimed process, since anthocyanin is separated in the retentate and sugars and acids are separated in the permeate, anthocyanin can be purified.

As illustrated in the attached Figure 1, the anthocyanin-containing composition of the present invention is never produced by the process proposed by Lawhon, since the fraction including anthocyanin without water, sugars and acids cannot be obtained by the process proposed by Lawhon.

Consequently, the teachings of Lawhon never contemplate or suggest a process to purify anthocyanin of the present invention, so as to obtain the presently claimed anthocyanin-containing composition in which anthocyanin is purified to be included in 5 to 25% by weight of black currant anthocyanin on the basis of solid matters. For all these reasons, applicant respectfully submits that it is impossible for the teachings of Lawhon to contemplate or suggest the presently claimed invention within the meaning of 35 U.S.C §102 or 35 U.S.C §103. Therefore, applicant respectfully request that the examiner reconsider and withdraw any and all rejections of the present claims over these teachings.

In the response filed on September 10, 2003, the meaning of "mg%" within the teachings of Nakhmedov was discussed. However, the outstanding Office Action does not mention this important teaching. For a better understanding of these teachings, the disclosures of Nakhmedov are explained again here and an English translation of Nakhmedov is attached hereto. Nakhmedov never discloses the composition including anthocyanin of the claimed composition of the present claims. The reference cited by the Patent Office uses the unit "mg%". However, the original literature in Russian does not use this unit. It uses the unit "mg/100g". The original literature in Russian and the English translation thereof, both of which are attached hereto, show this. Table 1 of Nakhmedov shows that approximately 800-2000 mg/100g of anthocyanin is contained in the pomace of black currant which is corresponding to 0.8 to 2% of anthocyanin. Accordingly, Nakhmedov never contemplates or discloses the presently claimed composition.

The teachings of the British '751 do not cure or rectify the deficiencies in the teachings of Nakhmedov. For example, the teachings of the British '751 do not contemplate or suggest black currant concentrate and, therefore, cannot contemplate or suggest specific amounts of black currant anthocyanin, as required in the present claims.

An advantage of the composition of the present claims is that it can be used as a food or a food additive. This is because the composition of the present claims has excellent stability against spoilage and reduced acidity. In this connection, it is respectfully noted that the teachings of Nakhmedov state

that the materials proposed therein were contaminated and spoiled easily, meaning it is not suitable as a food or a food additive. This statement in Nakhmedov evidences the fact that the composition proposed therein is different from that defined in the present claims.

The pharmacologic effects of anthocyanin other than those derived from black currant anthocyanin on peripheral arteries are the effect as pharmaceuticals not as foods. The presently claimed composition made it possible to utilize the pharmacologic effects of black currant anthocyanin in foods. Furthermore, the only previously known effect of anthocyanin was the effect on arteries. On the contrary, black currant anthocyanin of the present invention acts on blood to improve blood fluidity and lower blood pressure as the result of example 12 of the present specification shows.

For the reason set forth above, applicant respectfully submits that the present claims are distinguishable from the teachings of Lawhon, Nakhmedov, and/or British '751 within the meaning of 35 U.S.C. § 102 or 35 U.S.C. § 103. Therefore, applicant respectfully requests that the examiner reconsider and withdraw this rejection.

For the foregoing reasons, applicant respectfully requests that the examiner reconsider and withdraw all the objections and rejections set forth in the Official action mailed November 25, 2003, so that all pending claims 27-30 and 32-41 will be allowed.

While it is believed that the present application is in condition for allowance, should the examiner have any comments or questions, it is

respectfully requested that the undersigned be telephoned at the below listed number to resolve any outstanding issues.

In the event that this paper is not timely filed, applicant hereby petitions for an appropriate extension of time. The Commissioner is hereby authorized to charge the fee therefor, as well as any deficiency in the payment of the required fee(s) or credit any overpayment, to our deposit account No. 22-0256.

Respectfully submitted,
VARNDELL & VARNDELL, PLLC
(formerly Varndell Legal Group)

R. Eugene Varndell, Jr.
Registration No. 29,728

Atty. Case No. VX012397
106-A S. Columbus St.
Alexandria, Virginia 22314
(703) 683-9730
\\V:\VDOCS\W_DOCS\FEB05\P080-2397 PA.DOC

Attachments:
Figure 1, and
Nakhmedov *et al.* (Koservanaya I Ovoshchesushil'naya Promyshlennost) and English translation thereof.

Таблица 2

Ягоды	Концентрация сиропа при заливке, %	Начальная концентрация сиропа, %	Продолжительность кипения, мин	Остаточное давление, Па	Продолжительность, мин	Остаточное давление, Па	Продолжительность кипения, мин	Остаточное давление, Па
	Первая варка				Охлаждение		Вторая варка	
Вишня без косточки	60	55	15	$199 \cdot 10^2$	10	$532 \cdot 10^2$	15	$199 \cdot 10^2$
Черная смородина	70	60	10	$199 \cdot 10^2$	10	$532 \cdot 10^2$	15	$199 \cdot 10^2$

чества сахара по рефрактометру нуждаются в соответствующих поправках для определения истинного содержания сорбита в растворе.

Всесоюзным научно-исследовательским институтом кондитерской промышленности и Украинским научно-исследовательским институтом пищевой промышленности предложена формула расчета истинного содержания сухих веществ в растворах, содержащих сорбит, а также добавки других растворимых в воде веществ.

При расчете норм расхода сырья и материалов исходят из истинного содержания сухих веществ в готовом варенье.

Для этого применяют расчетную формулу, предложенную ВНИИКПом и УкрНИИППом [1],

$$B = [0{,}01\,C\,(1{,}1A - 3{,}9)] + 0{,}01\Pi A,$$

где A — показание рефрактометра при исследовании готового продукта;

C и Π — соответственно рецептурное содержание сорбита и плодов в пересчете на сухое вещество в % к сумме сухих веществ смеси ($C + \Pi = 100\%$);

0,1; 1,1 и 3,9 — постоянные коэффициенты, найденные эмпирически.

Первые партии диетического варенья из вишни изготовлены в 1974 г. на Симферопольском и Кодымском консервных заводах по технической документации, разработанной Украинским научно-исследовательским институтом консервной промышленности.

Согласно действующим техническим условиям содержание сухих веществ в готовом варенье должно быть не менее 68%, общего количества сахаров — не более 7%, содержание сорбита в 100 г варенья — 64—68 г.

[1] Ланьшина Т. С., Попова Ю. И., Лурье И. С. Определение сухих веществ в водных растворах сорбита и ксилита,— «Хлебопекарная и кондитерская промышленность», 1969, № 9, с. 17.

УДК [667.211.5 : (634.18 + 634.72)] : 664

Канд. биол. наук Ф. Г. НАХМЕДОВ,
канд. техн. наук М. Л. ФРУМКИН,
ст. техник В. А. СВИСТУНОВА,
Всесоюзный научно-исследовательский институт консервной и овощесушильной промышленности;
В. М. МЯЧИН,
директор Мичуринского консервного комбината

Красители из отходов переработки черноплодной рябины и черной смородины

В течение ряда лет нами исследовались химико-технологические и микробиологические особенности отходов производства консервов из темноокрашенных плодов и ягод с целью использования их для получения натуральных красителей.

Опыты проводили в лабораторных и полупроизводственных условиях в течение 1971—1973 гг. с отходами, образующимися при переработке черноплодной рябины и черной смородины. Сырье (по 35—40 кг каждого вида) получали из хозяйств Московской области и раздельно подвергали технологическим операциям (мойке, дроблению или протиранию, прессованию) на лабораторном оборудовании по принятым режимам получения натуральных соков, соков с мякотью и соков для изготовления плодово-ягодных вин.

После отделения сока выжимки разделяли на две части: одну оставляли в качестве выжимок первого отжима, а вторую заливали водой и через несколько часов вторично отпрессовывали для получения выжимок второго отжима. После взятия проб для химических и микробиологических анализов выжимки

первого и второго отжимов разделяли на две части и использовали для получения красителя: одну — сразу же после отделения сока, а другую — после хранения в течение 10 дней в негерметичной таре при комнатной температуре. В обоих случаях красители получали методом экстрагирования красящих веществ горячей водой.

Параллельно с лабораторными опытами проводили исследования по тем же режимам в производственных условиях на Мичуринском консервном комбинате.

Содержание антоцианов в выжимках и красителях определяли по модифицированной нами методике J. B. Harborne [1] М. К. Зейкела [2]. Антоцианы извлекали из выжимок 3%-ным раствором соляной кислоты в этаноле, а из красителя — 2%-ным раствором соляной кислоты в этаноле.

Определение содержания антоцианов проводили на фотоэлектроколориметре ФЭК-М с зеленым светофильтром и в кюветах шириной 10 мм. Для пересчета экстинкций используемого раствора пользовались калибровочной кривой, составленной на чистом препарате мальвидина.

Состав антоцианов определяли методами нисходящей и двумерной бумажной хроматографии с использованием растворителей: *н*-бутанол : уксусная кислота : ввода (4:1:1) и 2%-ная уксусная кислота. Высушенные хроматограммы проявляли водными растворами углекислого и уксуснокислого натрия. Идентифицировали антоцианы сопоставлением обнаруженных пятен с *Rf* на хроматограммах J. B. Harborne [1]. Содержание сухих веществ, сахаров, общую кислотность и pH определяли общепринятыми методами химического анализа.

При микробиологических исследованиях определяли общее количество бактерий методом счета колоний на мясопептонном агаре.

Плесневые грибы и дрожжи выявляли высевом смывов на сусло-агар. Посевы термостатировали при 28° С в течение 3 суток. Анализы проводили перед закладкой выжимок на хранение и периодически в течение всего срока хранения. 10—20 г пробы брали из каждого вида сырья.

Аналогичные микробиологические исследования проводили в изготовленных опытных партиях красителя.

Технологические опыты проводили ежегодно в пятикратной повторности. Химический анализ выжимок и красителей проводили на трех параллельных пробах, а микробиологический — на пяти. Полученные результаты обрабатывали для вычисления среднеарифметических величин.

Исследования показали, что отходы, образующиеся при переработке черноплодной рябины и черной смородины, содержат значительное количество сухих веществ и антоцианов, которые обусловливают получение из них натуральных пищевых красителей (табл. 1).

Как видно из приведенных в табл. 1 данных, чем выше содержание сухих веществ и антоцианов в выжимках, тем выше выход красителей. В выжимках первого отжима содержание сухих веществ и антоцианов в два с лишним раза больше, чем в выжимках второго отжима. Из выжимок первого отжима выход красителей с содержанием сухих веществ 40% составил 24—25%, а из выжимок второго отжима — лишь 10—11%.

Микробиологические исследования показали, что выжимки первого и второго отжимов сильно обсеменены разнообразной микрофлорой: споровыми палочками, дрожжами, неспоровыми палочками типа Bact. Herbicola,

Таблица 1

Выжимки	Количество выжимок, кг	Содержание в выжимках		Количество красителя, полученного из выжимок		
		сухих веществ, %	антоцианов, мг на 100 г	кг	%	содержание антоцианов в красителе, мг на 100 г
Из черноплодной рябины						
Первого отжима	38,5	10,8±0,2	1723,5±15,5	9,82	25,5±1,5	5235,4±15,3
Второго отжима	30,4	6,8±0,4	925,3±14,8	3,5	11,5±0,9	4924±10,0
Из черной смородины						
Первого отжима	40,0	11,9±0,5	2020,8±26,8	9,95	24,9±0,3	6256,8±11,5
Второго отжима	40,0	5,4±0,3	874,7±26,5	4,1	10,25±2,0	6128,9±15,2

Proteus, пигментированными и непигментированными кокками, лактобациллами и меньше плесенями (табл. 2).

Как видно из данных табл. 2, в первый день закладки на хранение ягоды, свежеотжатый сок и выжимки были обсеменены микрофлорой почти одинаково. Однако уже на третий день хранения при комнатной температуре сок начал бродить, на ягодах появились признаки плесневения, а выжимки остались почти без изменения. К концу хранения на десятый день ягоды имели явный признак порчи, в соке наблюдалось интенсивное брожение, на поверхности выжимок был лишь тонкий слой плесени. При этом выявилось, что выжимки из черной смородины быстрее плесневеют, чем из черноплодной рябины.

Стабильность выжимок к микробиальной порче при хранении можно объяснить, видимо, тем, что они в несколько раз беднее сахарами и другими питательными веществами, необходимыми для роста и развития микроорганизмов, чем ягоды и соки. Кроме того, в выжимках значение pH значительно ниже (~2,9—3,2), чем в ягодах и соке (~4,0—4,2). Благодаря этому рост и развитие дрожжей и плесеней в выжимках протекают замедленно.

Установлено, что наиболее приемлемыми сроками переработки выжимок на красители являются первые три дня после их получения. Выжимки, обсемененные спонтанной микрофлорой и заплесневевшие более чем на 30%, дают красители с темно-бурой окраской, вареным (с запахом плесени) привкусом, более высоким pH и низким содержанием антоцианов, чем выжимки с незначительной степенью обсемененности (табл. 3).

Таблица 2

Исследуемый продукт	Количество микроорганизмов в 1 г до хранения сырья	
	всего	в том числе дрожжей и плесеней
Черноплодная рябина		
Ягоды	$3,4 \cdot 10^5$	$2,1 \cdot 10^3$
Сок	$1,5 \cdot 10^{6*}$	$1,2 \cdot 10^{3*}$
Выжимки	$2,5 \cdot 10^6$	$2,1 \cdot 10^3$
Черная смородина		
Ягоды	$2,1 \cdot 10^6$	$3,1 \cdot 10^4$
Сок	$1,3 \cdot 10^{4*}$	$1,1 \cdot 10^{4*}$
Выжимки	$3,4 \cdot 10^6$	$1,5 \cdot 10^3$

* В 1 мл.

Как видно из данных табл. 3, в красителях, полученных из выжимок, не имеющих признаков порчи, содержание антоцианов, органических кислот и сахаров почти в 2 раза больше, чем в красителях из заплесневевшего сырья. Это объясняется, по-видимому, тем, что дрожжи и плесени для сохранения своей жизнедеятельности и интенсивного размножения расходуют не только сахара и органические кислоты, но и антоциановые пигменты. Возможно также, что снижение антоцианов в красителях из сильно заплесневевших выжимок вызвано повышением значения pH, так как при изменении величины pH происходит перегруппировка

Таблица 3

Показатели	Красители из выжимок черноплодной рябины		Красители из выжимок черной смородины	
	свежих	заплесневевших	свежих	заплесневевших
Содержание антоцианов, мг на 100	6328,3±18,1	2845,6±14,3	7125,4±19,2	3129,9±11,9
pH	3,5±0,2	5,1±0,5	3,2±0,3	4,8±0,5
Общая кислотность, %	6,9±0,4	4,8±0,3	9,8±0,2	5,4±0,2
Содержание сахаров, %	15,3±0,2	12,4±0,4	16,1±0,7	13,3±0,8
Внешний вид	Густая сиропообразная жидкость, вязкая	Менее вязкая, сиропообразная жидкость	Густая сиропообразная жидкость, вязкая	Менее вязкая сиропообразная жидкость
Вкус	Слегка терпкий, свойственный ягодам	С посторонним привкусом, слабой горечью	Кислый	Менее кислый с посторонним привкусом
Запах	Свойственный ягодам черноплодной рябины	Плесени	Приятный, свойственный ягодам черной смородины	Плесени
Цвет	Темно-красный	Темно-бурый	Темно-красный	Темно-бурый
Обсемененность микроорганизмами, клеток в 1 мл	0	$1,5 \cdot 10^3$	0	$1,5 \cdot 10^4$

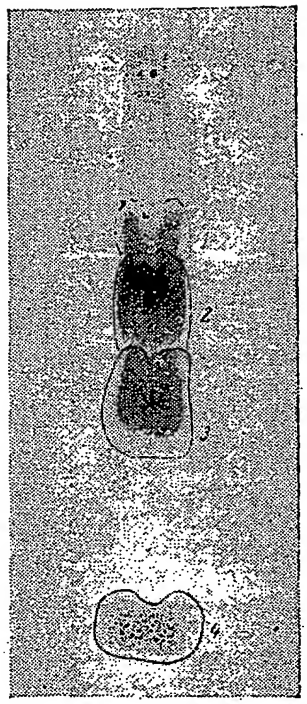


Рис. 1. Хроматограмма антоцианов красителя из выжимок черноплодной рябины:
1 — дельфинидин; 2 — цианидин; 3 — цианидин-3-глюкозид; 4 — цианидин-3,5-диглюкозид.

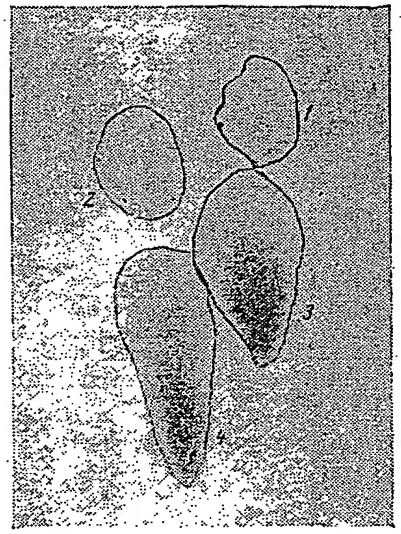


Рис. 2. Хроматограмма антоцианов красителя из выжимок черной смородины:
1 — цианидин; 2 — дельфинидин; 3 — цианидин-3-глюкозид; 4 — дельфинидин-3-рутинозид.

в структуре антоцианов и образование свободных агликонов и остатков сахаров или новых форм фенольных соединений.

Таким образом, из выжимок, обсемененных большим количеством микроорганизмов, а также имеющих признаки порчи, невозможно получить пищевой краситель с высокой красящей способностью, физико-химическими и органолептическими показателями. Свежие выжимки, содержащие около 10^2—10^4 клеток микроорганизмов в 1 г, пригодны для получения пищевых красителей, так как применяемые технологические режимы переработки выжимок и получения красителей позволяют получить готовый продукт стерильным с высокими качественными показателями.

Изучение состава антоциановых пигментов в красителях из свежих выжимок показало, что в красителях черноплодной рябины и черной смородины содержится 4 антоциановых пигмента.

Как видно из рис. 1, антоцианы красителя из выжимок черноплодной рябины представлены главным образом цианидином, цианидин-3-глюкозидом и цианидин-3,5-диглюкозидом. Наряду с этим в нем обнаружены также следы дельфинидина.

В отличие от красителя из выжимок черноплодной рябины на хроматограммах красителя из выжимок черной смородины более интенсивными оказались не только пятна цианидиновых пигментов, но и дельфинидина и дельфинидин-3-рутинозида (рис. 2).

В красителях из выжимок черноплодной рябины и черной смородины найдены почти все антоциановые пигменты, содержащиеся в плодах и ягодах.

Таким образом, свежеотжатые выжимки черноплодной рябины и черной смородины являются ценными источниками получения натуральных пищевых красителей.

Дополнительная прибыль от использования только 200 т отходов составит около 72 тыс. руб. в год.

СПИСОК ИСПОЛЬЗОВАННОЙ ЛИТЕРАТУРЫ

1. Harborne J. B. The Chromatographic identification of anthocyanin pigments.— "Jornal Chromatography", 1958, v. 1, p. 473.

2. Зейкел М. К. Выделение и идентификация фенольных соединений в биологических материалах.— В кн. Биохимия фенольных соединений. Под ред. Дж. Харборна. М., 1968, с. 34—49.

UDK [667.211.5: (634.18 + 634.72)]: 664

Nakhmedov F.G., Ph.D. (biology),
Frumkin M.L., Ph.D. (engineering),
Svistunova V.A., senior technician,
The All-Union Research Institute for Canning and Vegetable-Drying Industry;
Myachin V.M.,
Director of Michurinsk canning combinat

Coloring agents from the wastes of chokeberry and black currant processing

Over a period of years we have been researching chemico-technological and microbiological characteristics of the wastage of canned foods of dark-colored fruits and berries, with the purpose to use them for production of natural colors.

Experiments were carried out in laboratory and semi-plant conditions during 1971-1973 years with the wastes formed in processing of chokeberry and black currant. Raw material (35-40 kg of each type), delivered from the farms of Moscow Region, was separately subjected to process operations (washing, crushing or rubbing, pressing) on the laboratory equipment according to generally accepted conditions for the production of natural juices, juices with pulp and juices for manufacturing of fruit wines.

After separation of juice, the pressed skins were divided into two parts: one was left as the marc of the first pressing, and the second was poured with water and in a few hours pressed again for

receiving secondarily pressed marcs. After sampling for chemical and microbiological analyses, the firstly and secondarily pressed marcs were divided into two parts and used for production of colors: one immediately after separation of juice, another after ten-days storage in unsealed container at room temperature. In both cases colors were obtained by extracting the coloring agents with hot water.

In parallel with the laboratory tests, experiments under the same conditions with manufacturing ones were carried out at Michurinsk canning combinat.

The content of anthocyanins in marcs and colors was determined by the method of J. B. Harborne [1] and M. K. Zeikel [2] modified by authors. Anthocyanins were extracted from the marcs with a solution of 3% hydrochloric acid in ethanol, and from colorants with a solution of 2% hydrochloric acid in ethanol.

The anthocyanins content was determined on an FEK-M photoelectrocolorimeter with green filter and in a cell of 10 mm thick. Calibration curve prepared based on pure malvidine preparation was used for conversion of extinction of the solution to be used.

Composition of the anthocyanins was determined by the methods of descending and two dimensional paper chromatography using the following solvents: n-butanole : acetic acid : water (4 : 1 : 1) and 2%-acetic acid. Dry chromatograms were developed by the aqueous solutions of sodium carbonate and sodium acetate. Anthocyanins were identified by comparison of the developed spots with *Rf* on J. B. Harborne [1]. The content of solid matters and sugars, the total acidity and the pH value were determined by generally accepted methods of chemical analysis.

In microbiological experiments, the total number of bacteria was determined by counting the colonies on beef-peptone agar. Mold fungus and yeast were detected by sowing the wash-out solution on wort agar. The inoculaums were maintained at 28 °C during 3 days. The analyses were carried out before depositing marc for storage and periodically during the whole storage period. Samples of 10-20 g were taken from each type of raw materials.

Similar microbiological experiments were carried out for produced colorants.

Process examinations were carried out periodically five times a year. Chemical analysis of marcs and colors was made with three parallel samples, and microbiological analysis - with five samples. Thus obtained results were processed for calculation of arithmetic mean values.

The experiments indicated that the wastes resulting from chokeberry and black currant processing include a significant amount of solid matters and anthocyanins, which preconditions manufacturing of natural food coloring agents from them (Table 1).

Table 1

Marcs	Marc quantity, kg	Content in marcs		Quantity of coloring agent produced from marc		
		dry substances, %	anthocyanins, mg per 100 g	kg	%	content of anthocyanins in coloring agent, mg per 100 g
From the chokeberry						
of the first pressing	38.5	10.8 ± 0.2	1723.5 ± 15.5	9.82	25.5 ± 1.5	5235.4 ± 15.3
of the second pressing	30.4	6.8 ± 0.4	925.3 ± 14.8	3.5	11.5 ± 0.9	4924 ± 10.0
From the black currant						
of the first pressing	40.0	11.9 ± 0.5	2020.8 ± 26.8	9.95	24.9 ± 0.3	6256.8 ± 11.5
of the second pressing	40.0	5.4 ± 0.3	874.7 ± 26.5	4.1	10.25 ± 2.0	6128.9 ± 15.2

As may be seen from the data in Table 1, the higher is the content of solid matters and anthocyanins in the marc, the higher is the yield of the colorants. In the marc of the first pressing the content of solid matters and anthocyanins is two times higher than in marc of the second pressing. Of the marcs of the first pressing, the yield of colorants containing 40% of solid matters amounts to 24-25%, and, of the marcs of the second pressing, the yield - only to 10-11%.

Microbiological experiments showed that the marcs of first and second pressing are heavily bacterized with various microflora: spore bacillus, yeast, non-spore bacillus of Bact. Herbicola type, Proteus, pigmented and not pigmented coccus, lactobacillus and to lesser extent molds (Table 2).

As seen from the data in Table 2, on the first day of depositing berries in storage, newly pressed juice and marcs were sown with microflora almost equally. However on the third day of storage at room temperature, the juice began fermentation; there appeared

evidences of mold growth on berries, while marcs remained almost unchanged. At the end of storage on the tenth day, there appeared an obvious evidence of spoilage in the berries, intense fermentation in the juice, and only a thin layer of molds on the surface of marcs. At the same time, it was discovered that marcs of black currant got moldy quicker than marcs of chokeberry.

Table 2

Product to be tested	Quantity of microorganisms in 1 g before storage of raw material	
	Total	Including yeast and molds
	Chokeberry	
Berries	$3.4 \cdot 10^5$	$2.1 \cdot 10^3$
Juice	$1.5 \cdot 10^6$*	$1.2 \cdot 10^3$*
Marc	$2.5 \cdot 10^6$	$2.1 \cdot 10^3$
	Black currant	
Berries	$2.1 \cdot 10^6$	$3.1 \cdot 10^4$
Juice	$1.3 \cdot 10^4$*	$1.1 \cdot 10^4$*
Marc	$3.4 \cdot 10^6$	$1.5 \cdot 10^2$

* In 1 ml

Resistance of the marcs to microbiological spoilage during storage may be probably explained by the fact that they are several times poorer in sugars and other nutrients necessary for growth and development of microorganisms, than berries and juices. Besides, the pH value in the marc is significantly lower (~2.9-3.2) than that of berries and juices (~4.0-4.2). Thereby the growth and development of yeast and molds in the marcs proceed slowly.

It is determined that the most acceptable terms of processing the marc into colorants are the first three days after their production.

The marcs sown with the spontaneous microflora and grown moldy to more than 30%, yield such colorant of dark-brown coloration, boiled flavor (smell of mold) higher pH and lower content of anthocyanins than that of marcs with an inconsiderable extent of bacteria growth (Table 3).

Table 3

Parameters	Colors from chokeberry marc		Colors from black currant marc	
	Fresh	molded	fresh	molded
Content of anthocyanins, mg per 100:	6328.3 ± 18.1	2845.6 ± 14.3	7125.4 ± 19.2	3129.9 ± 11.9
pH	3.5 ± 0.2	5.1 ± 0.5	3.2 ± 0.3	4.8 ± 0.5
Total acidity, %	6.9 ± 0.4	4.8 ± 0.3	9.8 ± 0.2	5.4 ± 0.2
Content of sugars, %	15.3 ± 0.2	12.4 ± 0.4	16.1 ± 0.7	13.3 ± 0.8
Appearance	Thick syrup-like liquid, viscous	Less viscous syrup-like liquid	Thick syrup-like liquid, viscous	Less viscous syrup-like liquid
Taste	Slightly acerbic, peculiar to berries	With foreign flavor and slight bitterness	Sour	Less sour with off-flavour
Odour	Peculiar to berries of chokeberry	Of mold	Pleasant, peculiar to berries of black currant	Of mold
Color	Crimson	Dark brown	Crimson	Dark brown
Bacteria growth with microorganisms, cells in 1 ml	0	$1.5 \cdot 10^2$	0	$1.5 \cdot 10^4$

"Canning and vegetable-drying industry", № 4

As seen from the data in Table 3, in the colors produced from the marc without any sign of spoilage, the content of anthocyanins, organic acids and sugars is almost two times higher than in colors from the molded raw materials. This may be explained presumably by the fact that yeast and molds, in order to maintain their viability and intense generation, consume not only sugars and organic acid, but also anthocyanin pigments. Also, the decrease of anthocyanins in the colors from strongly molded marcs is probably induced by the increase of pH value, since when pH value is changed, there occur rearrangement

in anthocyanins structure and formation of free aglycone and of the residues of sugars or new forms of phenolic compounds.

Thus, from the marcs sown with a lot of microorganisms and showing any sign of spoilage, it is impossible to obtain such a food color with a high coloring capacity, physicochemical and organoleptic characteristics. Fresh marcs containing about 10^2-10^4 of microorganism cells in 1 g are suitable for making food colorants, since technological conditions used for marc processing and colorants manufacturing allow sterile end products of high quality to be obtained.

The study about the content of anthocyanin pigments in colorants made of the fresh marcs showed that chokeberry and black currant colors contain 4 anthocyanin pigments.

As seen from Figure 1, anthocyanins of the colorant obtained from chokeberry marc are presented mainly by cyanidine, cyanidine-3-glucoside and cyanidine-3,5-diglucoside. At the same time the traces of delphinidine were found in it.

In contrast to the colorant obtained from chokeberry marcs, chromatograms of the colorant from black currant marcs showed that not only spots of cyanidine pigments, but also spots of delphinidine and delphinidine-3-rutinoside were more intense (Figure 2).

Figure 1. Chromatogram of anthocyanins in the colorant of chokeberry marc:
1 - delphinidine; 2 - cyanidine; 3 - cyanidine-3-glucoside; 4 - cyanidine-3,5-diglucoside.

Figure 2. Chromatogram of anthocyanins in the colorant of black currant marc:

1 - cyanidine; 2 - delphinidine; 3 - cyanidine-3-glucoside; 4 - delphinidine-3-rutinoside.

Almost all anthocyanin pigments contained in fruits and berries were discovered in the colorants made of chokeberry and black currant marcs.

Consequently, newly pressed marcs of chokeberry and black currant are a valuable source for production of natural food colors.

Additional profit from only 200 tons of wastes used will amount to approx. 72 thousand roubles per year.

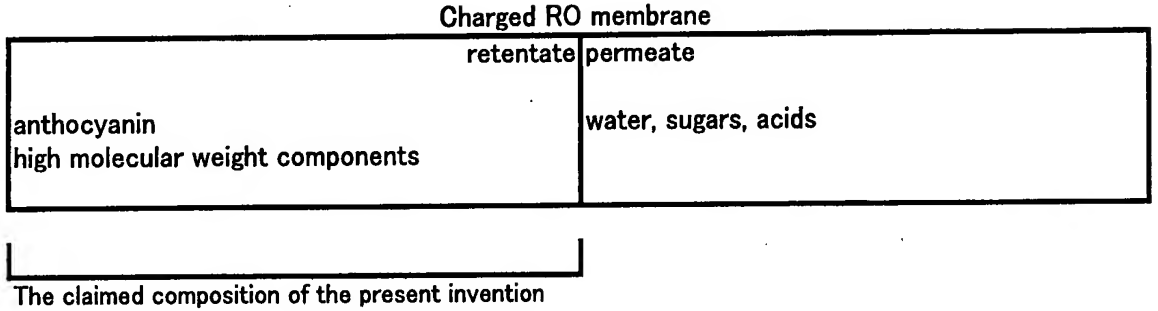
LITERATURE

1. Harborne J.B. The Chromatographic identification of anthocyanin pigments. - "Journal Chromatography", 1958, v. 1, p. 473.

2. Zeikel M.K. Separation and identification of phenolic compounds in biological materials. - in the book "Biochemistry of phenolic compounds", by Harborne J., Moscow, 1968, p. 34-49.

Figure 1

The process of the present invention



Lawhon's process